A STUDY OF THE SULPHUR METABOLISM OF WHEAT, BARLEY AND CORN USING RADIOACTIVE SULPHUR[1]

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(WITH TWO FIGURES)

Radioactive sulphur (S^{35}) with its weak beta radiation of 0.120 M.E.V. and its long half-life of 87 to 88 days (6, 8) has desirable characteristics as a biological tracer, particularly since its activity can be readily measured after recovery as barium sulphate (6). Further, as will be shown in the following paper (5), excellent autographs of thin sections of plant material can be made on photographic film.

SEABORG (9) and HAMILTON (3, 4) have reviewed the literature up to 1941 on the use of radioactive elements as tracers in chemical and biological work. Radiosulphur in synthetic cystine, methionine, and thiamin as well as inorganic compounds, has been used in nutritional studies of animals (1, 10), but so far as is known, nutritional studies of plants using radiosulphur have not been reported.

This paper describes experiments carried out in 1942 with radiosulphur furnished as barium sulphate*[2] by Dr. J. G. HAMILTON of the Radiation Laboratory, University of California. The sulphur* was added to vegetation growing in the large sand cultures (13) both as a soluble sulphate* in the nutrient solution and as sulphur* dioxide in the air. No radiosulphur was available during the 1943 season.

Methods and results

DESCRIPTION OF THE PLOTS

Six plots of mixed wheat and barley were treated between June 6, and June 30, 1942, three with sodium sulphate*, and three with sulphur* dioxide. A similar treatment was applied to a pair of Bancross corn plots on Sept. 16 to 18. The principal agronomic details of these plots are summarized in table I. Each of the wheat and barley plots had two rows of Federation spring wheat, one row of Velvon barley, and two rows of no. 54A–40 winter wheat supplied by the Utah Experiment Station. The winter wheat remained in the vegetative state until harvest, tillering continuously. The barley and spring wheat plants began to head out during the first week of June. They began to flower about the middle of June, and their lower leaves and beards began to dry up at the end of June. The barley was a few days ahead of the wheat in all these processes. Ripening was well advanced, particularly of the primary heads, by the middle of July and the plots were harvested

[1] This is the sixth of a series of papers on "The Effect of Prolonged Low Concentrations of Sulphur Dioxide Upon Plants."

[2] The asterisk following sulphur refers to active preparations containing sulphur of atomic weight 35.

TABLE I

DESCRIPTION OF PLOTS TREATED WITH RADIOSULPHUR

PLOT NO.	DATE OF PLANTING	DATE OF TREATMENT 1942	STAGE OF GROWTH	NUTRIENT SOL.†			SULPHUR* DIOXIDE FUMIGATION††			HARVEST DATA					
										DRY WEIGHT					
				CONC. LEVEL	pH	SULPHATE S.	DURATION	TOTAL CONC.	ABSORPTION‡	DATE	GRAIN	TOPS	ROOTS	TOTAL	TOTAL CROPS
						p.p.m.	*min.*	*p.p.m.*	%	*July*	*Kg.*	*Kg.*	*Kg.*	*Kg.*	*Kg.*
WHEAT AND BARLEY															
B-4	3-24	6-6	Heading out	High	6.8	0.47	158	0.10	52	(1) 29	0.43	0.64	0.08	1.15	3.59
										(2) 30	0.17	0.51	0.03	0.71	
										(3) 31	....	1.55	0.17	1.73	
B-2	3-24	6-23	Blossom	High	6.8	0.47	....	....	....	(1) 29	0.30	0.53	0.06	0.89	2.56
										(2) 30	0.08	0.39	0.03	0.50	
										(3) 31	....	0.95	0.22	1.17	
A-3	3-24	6-22	Blossom	Low	6.0	0.49	178	0.08	40	(1) 28	0.44	0.47	0.06	0.97	2.80
										(2) 30	0.17	0.25	0.02	0.44	
										(3) 31	....	1.12	0.27	1.39	
A-1	3-24	6-23	Blossom	Low	6.1	0.43	....	....	....	(1) 28	0.32	0.36	0.06	0.74	2.02
										(2) 30	0.15	0.21	0.03	0.39	
										(3) 31	....	0.71	0.18	0.89	
B-3	3-24	6-29	Milk	High	5.8	0.46	143	0.07	55	(1) 29	0.38	0.57	0.08	1.03	3.43
										(2) 30	0.22	0.42	0.04	0.73	
										(3) 31	....	1.46	0.21	1.67	
B-1	3-24	6-30	Milk	High	5.8	0.44	....	....	....	(1) 29	0.35	0.58	0.04	0.97	2.70
										(2) 30	0.10	0.44	0.06	0.60	
										(3) 31	....	0.94	0.19	1.13	
CORN										*Oct.*					
D-4	8-18	9-18	Initial	High	6.9	5.55	143	0.18	68	31	0.02	0.80	0.10	0.92	
D-2	8-18	9-16	Initial	High	6.9	5.60	....	....	....	31	0.04	0.80	0.11	0.95	

† Volume of solution 1900 liters.
†† See text, for summary of fumigations with inactive sulphur dioxide.
‡ Approximate.
(1) Spring wheat.
(2) Barley.
(3) Winter wheat.

between July 28 and 31. The roots were removed Aug. 13 to 15. Some of the plants produced new leaves after the harvest of the tops. The corn plots were planted on Aug. 18, and the plants were still small but growing rapidly when the radiosulphur treatment was applied.

The wheat and barley plots used in the experiment were all supplied with sulphate-deficient nutrient solutions. In one pair of plots the pH of the nutrient solution was 6.8; in the other two pairs the pH was 5.8 to 6.1. Further, in one pair of plots the average general nutrient level was 12 per cent. and in the other two pairs it was 40 per cent. of HOAGLAND's concentration (7). In contrast to the wheat-barley plots, the corn was supplied with an adequate amount of sulphate in the nutrient solution.

As a part of the experimental conditions to which the plots considered in this paper (as well as the remainder of the 16 which comprise the complete installation) were subjected, half of the wheat-barley plots were fumigated with inactive sulphur dioxide for 5 hours a day on 83 days between April 14 and July 28, or a total of 413 hours of fumigation. The average concentration was 0.044 p.p.m. Similarly, half the corn plots had fumigations on 46 days between Sept. 3 and Oct. 29, for a total of 239 hours. The average concentration was 0.10 p.p.m. and the average daily duration 5.2 hours. Fumigations were usually applied between 9:30 A.M. and 2:30 P.M. Only fumigated plots were treated with sulphur* dioxide and only non-fumigated plots with sodium sulphate*.

Analyses of the wheat and barley plants for total sulphur at harvest time are summarized in table II.[3] In view of previous experience with alfalfa (12) the increase of total sulphur due to fumigation with sulphur dioxide in these experiments was surprisingly small. In some parts of the plants the unfumigated material had a greater concentration of total sulphur than the corresponding fumigated material. Similar data for corn in table VI also shows only a small effect due to fumigation. Evidently absorption of sulphur dioxide is less rapid and translocation from the leaves more rapid in these crops than in alfalfa. The fumigated crops were larger than the corresponding unfumigated crops (table I). The total amount of sulphur in them was therefore considerably greater also.

PREPARATION AND APPLICATION OF SODIUM SULPHATE* AND SULPHUR* DIOXIDE

Part of the radioactive barium sulphate* was fused with a slight excess of sodium carbonate. All the activity was leached from the melt with water, giving a solution of sodium sulphate* of which aliquot portions were added to the nutrient solutions of the sand culture plots. The remainder of the barium sulphate* was heated at 900° to 1000° C. in an atmosphere of dry hydrogen (2). Most of the sulphate* was reduced to sulphide* but a small amount of hydrogen sulphide* was evolved which was received in 0.1 N iodine dissolved in normal potassium iodide. When the reaction in the furnace was complete, the barium sulphide* was added to the iodine solution.

[3] The sulphur analyses in this paper were carried out by T. R. COLLIER.

The latter was warmed to coagulate the precipitated sulphur*, which was filtered off and heated at 115° C. in an autoclave to render it soluble in carbon disulphide. An aliquot portion of the carbon disulphide solution of sulphur* was evaporated in a small glass bulb attached to the intake tube of a 160-ml.

TABLE II

TOTAL SULPHUR IN THE WHEAT AND BARLEY PLANTS AT HARVEST

SAMPLE	FUMIGATED PLOTS				UNFUMIGATED PLOTS			
	B-4	A-3	B-3	Av.	B-2	A-1	B-1	Av.
	%	%	%	%	%	%	%	%
SPRING WHEAT								
Primary † grain	0.20	0.17	0.19	0.183	0.15	0.16	0.18	0.162
Secondary grain	0.17	0.17	0.19	0.173	0.16	0.16	0.17	0.163
Primary chaff	0.08	0.08	0.10	0.087	0.08	0.09	0.10	0.092
Secondary chaff	0.16	0.12	0.22	0.168	0.16	0.10	0.25	0.172
Primary straw	0.08	0.10	0.10	0.096	0.06	0.10	0.15	0.102
Secondary straw	0.11	0.11	0.24	0.152	0.15	0.10	0.24	0.162
Crowns	0.10	0.10	0.11	0.105	0.13	0.15	0.11	0.128
Roots	0.16	0.16	0.16	0.160	0.17	0.19	0.20	0.185
BARLEY								
Primary grain	0.14	0.13	0.13	0.136	0.13	0.13	0.13	0.130
Secondary grain	0.16	0.15	0.16	0.157	0.15	0.13	0.16	0.147
Tertiary grain	0.18	0.17	0.16	0.171	0.18	0.16	0.18	0.175
Primary chaff	0.08	0.11	0.07	0.087	0.07	0.10	0.09	0.088
Secondary chaff	0.07	0.12	0.08	0.089	0.10	0.09	0.09	0.092
Tertiary chaff	0.12	0.13	0.16	0.139	0.13	0.10	0.16	0.131
Primary straw	0.06	0.05	0.05	0.064	0.04	0.06	0.05	0.051
Secondary straw	0.07	0.09	0.07	0.078	0.06	0.05	0.05	0.052
Tertiary straw	0.10	0.11	0.12	0.110	0.07	0.07	0.11	0.083
Quaternary straw	0.18	0.18	0.20	0.186	0.13	0.14	0.16	0.145
Crowns	0.12	0.10	0.13	0.116	0.14	Lost	0.13	0.135
Roots	0.16	0.23	0.18	0.192	0.18	0.17	0.20	0.183
WINTER WHEAT								
Senescent †† leaves	0.09	0.11	0.10	0.097	0.06	0.08	0.07	0.072
Old leaves	0.09	0.15	0.11	0.116	0.08	0.11	0.09	0.094
Intermediate leaves	0.14	0.18	0.15	0.157	0.09	0.14	0.13	0.120
Young leaves	0.16	0.19	0.18	0.177	0.13	0.16	0.18	0.156
Crowns	0.11	0.14	0.13	0.129	0.10	0.12	0.11	0.110
Roots	0.16	0.15	0.16	0.156	0.14	0.12	0.16	0.140

† "Primary," "Secondary," etc., refer to shoots of different ages.
†† "Senescent," "Old," etc., refer to leaves of different ages on the individual shoots.

mercury pipette. With air leaking slowly into the pipette, the tube connecting the pipette and bulb was heated gently with a flame and the bulb was heated gradually with another flame until the sulphur* was burned completely. The mixture of sulphur* dioxide and air in the pipette could then be added at any desired rate to the air stream passing through the plant chamber. Recovery of a considerable portion of the sulphur* dioxide not absorbed by the vegetation was accomplished by a special absorber attached to the outlet of the plant chamber.

Table III gives the dosage of radiosulphur used in the experiments and recovered in the vegetation and solutions. Weights of the element were calculated on the assumption that, since the counter window absorbed 50 per cent. of the radiation (**6**), each count represented two atoms of S^{35}. The unit of radioactivity is the "curie," defined as the radiation from one gram of

TABLE III

RADIOSULPHUR AS OF JULY 1, 1942, ADDED TO AND RECOVERED FROM THE PLOTS

Plot No.	Date of treatment	Initial concentration S^{35} in		Weight S^{35} as						
				$Na_2S^{35}O_4$			$S^{35}O_2$			
					Recovered in				Recovered in	
		Nutrient solution	Air	Added in solution	Plants	Nutrient solution	Added in air	Absorbed in cabinet calculated†	Plants	Nutrient solution
		parts per billion-billion		*micromicrograms*				*micromicrograms*		
B-4	6-6	....	0.5	......	....	....	84	44	30	0.7
B-2	6-23	32	......	61	52	2.4	....	....	....	....
A-3	6-22	....	0.4	......	....	....	84	34	31	0.7
A-1	6-23	32	......	61	49	1.3	....	....	....	....
B-3	6-29	....	0.6	......	....	....	84	46	44	0.8
B-1	6-30	32	......	61	50	1.4	....	....	....	....
D-4	8-18	....	0.13	......	....	....	20	14††	0.1	1.3
D-2	8-16	29	......	56	3.9	43.	....	....	....	....

† Approximate.
†† 3.3 micromicrograms found in nutrient solution Sept. 21.

radium and equal to 3.7×10^{10} disintegrations per second. The following relations were used:

1 microcurie = 32.3 micromicrograms of S^{35} = 18,500 counts per second.

1000 counts per second = 1.75 micromicrograms S^{35}.

Since it was possible to measure with considerable assurance, activity in the prepared samples of less than 0.05 counts per second above background, the addition of less than three microcuries to the nutrient solution gave a fairly satisfactory experiment, though a larger dosage would have been useful, particularly in making radioautographs (**5**). The delicacy and utility of this new tool is illustrated forcefully in table III.

The attenuation of the radiosulphur as applied in these experiments was very great. Its initial concentration in the nutrient solution was about 32 parts per billion billion. About 80 per cent. of this addition was recovered in the sulphur deficient wheat and barley plants. Only 8 per cent. was recovered in the "high sulphur" corn plants; most of the remainder was found in the nutrient solution. Evidently the active material was absorbed along with the inactive sulphur in accord with the demand of the vegetation for this element. Similarly the concentration of the $S^{35}O_2$ molecules was about 0.5 parts per billion billion parts of air. This was added along with about 0.1 p.p.m. of inactive sulphur dioxide to a much smaller-than-usual air stream passing through the cabinet. Approximate absorption values for sulphur dioxide were obtained by analysis of the air stream before and after contact with the vegetation (table I). Assuming that the same fraction of the sulphur* dioxide as of the inactive compound was absorbed by the vegetation and walls of the cabinet, a large part of the activity so absorbed was recovered in the wheat and barley, but only 1 per cent. in the corn. The weather was cold and windy on Sept. 18, and the walls of the greenhouse were wet during the fumigation, causing a large absorption by the walls. This fact, together with the lower concentration of sulphur* dioxide, and the limited amount of vegetation, accounts for the small absorption by the latter. An appreciable part of the sulphur* absorbed by the chamber was found three days later in the nutrient solution. In all cases a small amount of activity was unaccounted for in the plants and solutions. Presumably there was some absorption by the sand.

Recovery of radiosulphur in the vegetation

Starting shortly after the addition of the radiosulphur to the plots and continuing until harvest, samples of the vegetation were removed at intervals from the plant chambers for analysis. For the most part these samples were taken from the winter wheat, since more of this material was available than of the spring wheat and barley. A fairly complete series of corn leaf samples was also taken. Ten to fifteen active wheat leaves of intermediate age, weighing 6 to 8 grams, or an equal weight of corn leaves were usually chosen. An effort was made to have the successive samples as nearly comparable as possible. They were immediately ground up in a Waring blendor and subjected to mild alkaline digestion for labile sulphur analysis (11). After removal of the labile sulphur the reaction mixture was analyzed for sulphate, acid soluble organic, and acid insoluble organic sulphur. The labile sulphur came largely from cystine and closely related compounds; the acid soluble organic fraction probably contained methionine as an important constituent, but no definite information is available as to other organic sulphur compounds.

At harvest the plants were divided into many parts so that the various plant structures could be treated separately. These samples were all analyzed for total sulphur by the Parr bomb, and some of them—particularly the grain—were subjected to sulphur fractionation as well.

All analyses were carried out gravimetrically and the barium sulphate* activity was determined in the Geiger-Müller Counter, using 10-milligram aliquots of the precipitate (6). If a sample contained insufficient total sulphur* to yield 10 milligrams of barium sulphate*, a measured amount of sulphuric acid was added to the preparation before precipitation. The activity values were corrected for decay by calculating them to July 1 for the wheat and barley, and to Sept. 25 for the corn, except in table III, in which both groups of plots are calculated to July 1.

The question arose as to the possibility of exchange of radiosulphur atoms between the various compounds in the mixture, and an experiment was carried out in which a considerable amount of active sodium sulphate* was digested for three days with inactive leaf material. No activity was found in the labile fraction; 2 per cent. was found in the insoluble organic; 3 per cent. in the soluble organic; and 95 per cent. in the sulphate fraction. The possibility of slight interaction is therefore not excluded, though part of the activity found in the two organic fractions may have been due to insufficient washing of the insoluble residue or incomplete sulphate* precipitation from the filtrate, respectively. It seems unlikely that the interaction could have been large enough in any case to have modified appreciably the conclusions drawn from these experiments. TARVER and SCHMIDT (10) found insignificant exchange between elemental sulphur*, and cystine or cysteine.

DISPOSITION OF THE ABSORBED SULPHUR* IN THE GROWING PLANTS

Typical data showing the course of the sulphur reaction in the fresh leaf samples following additions of sodium sulphate* and sulphur* dioxide are presented in figure 1 for winter wheat (plots B–1 and B–3) and in figure 2 for corn (plots D–2 and D–4).

Absorption of the sodium sulphate* was rapid in the wheat. Maximum concentration in the leaves was found in plot B–1 in 8 days, but the corn plot D–2 required a longer time. Analysis of the nutrient solution of plot B–1 indicated the loss from it of 75 per cent. of the added activity in 8 days and 94 per cent. in 16 days. The nutrient solution of the corn plot (D–2) still retained 77 per cent. of its initial activity after 42 days. Absorption of sulphur* dioxide, on the other hand, all occurred during the 2.5-hour fumigation period and therefore the earliest samples of the leaves had the maximum concentrations. The differences in behavior between the wheat and the corn reflect principally the difference in sulphate content of the nutrient solutions since the wheat was growing under conditions of sulphur deficiency whereas the corn had an adequate supply of sulphur. Particularly it may be noted that the corn required a much longer time than the wheat for the concentration of active sulphur* absorbed from the nutrient solution to exceed that absorbed as sulphur* dioxide, even though the ratio of sodium sulphate* to sulphur* dioxide eventually absorbed by the corn plants was greater than in the wheat and barley.

Figures 1 and 2 indicate that there was a rapid change of the absorbed sulphate* and sulphur* dioxide to organic forms. Within 5 hours in the wheat, and within 1 to 2 hours in the corn, appreciable quantities of organic sulphur* were found. Presumably still earlier samplings would also have revealed this effect. In the wheat leaves, the organic sulphur* was approximately equally divided between the three fractions, and only a very small

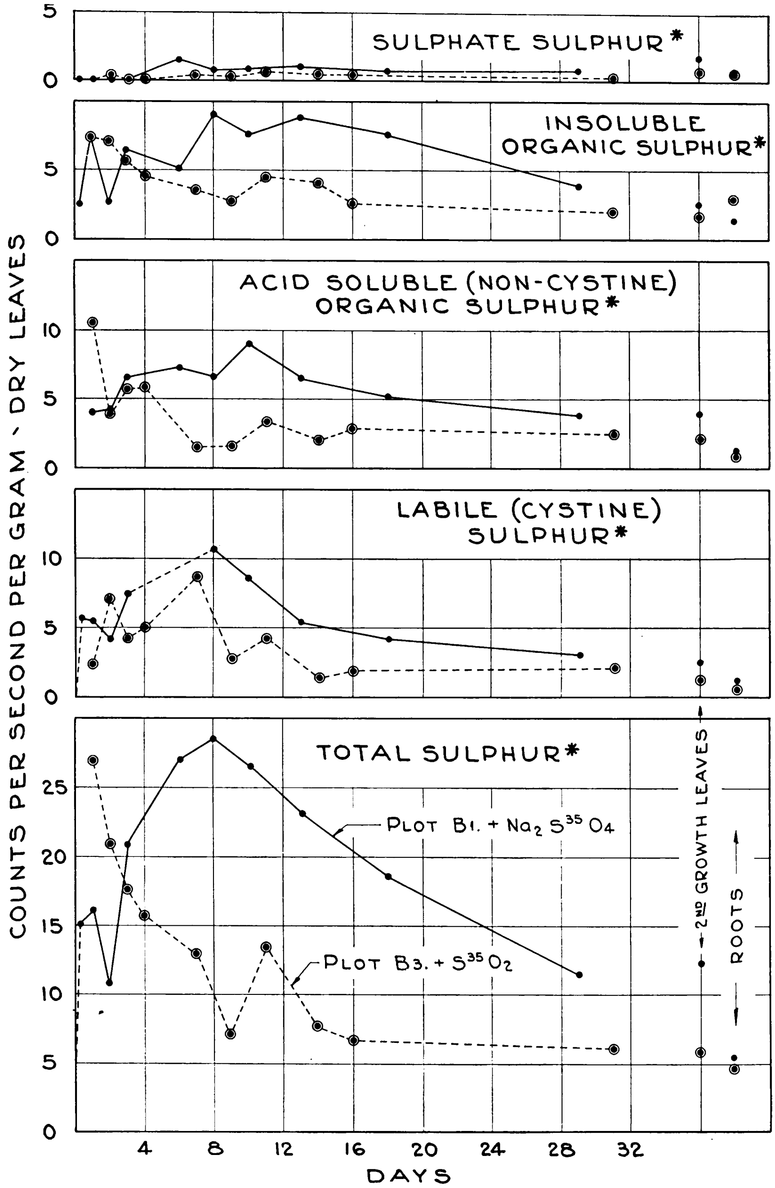

FIG. 1. Radiosulphur in the leaves of sulphur-deficient, vegetative winter wheat at various times after treating plots B–1 and B–3 with sodium sulphate* and sulphur* dioxide, respectively. Fractionation of the sulphur* into sulphate* and 3 organic sulphur* fractions is shown. The roots are also analyzed.

amount of sulphate* was present. The new leaves that grew in the period between harvest of the tops and the roots were similar in composition to the earlier leaves. In the corn plot D–4, sulphate* was the predominant constituent of the system for several days after the fumigation treatment. Thereafter its concentration was approximately equal to those of soluble

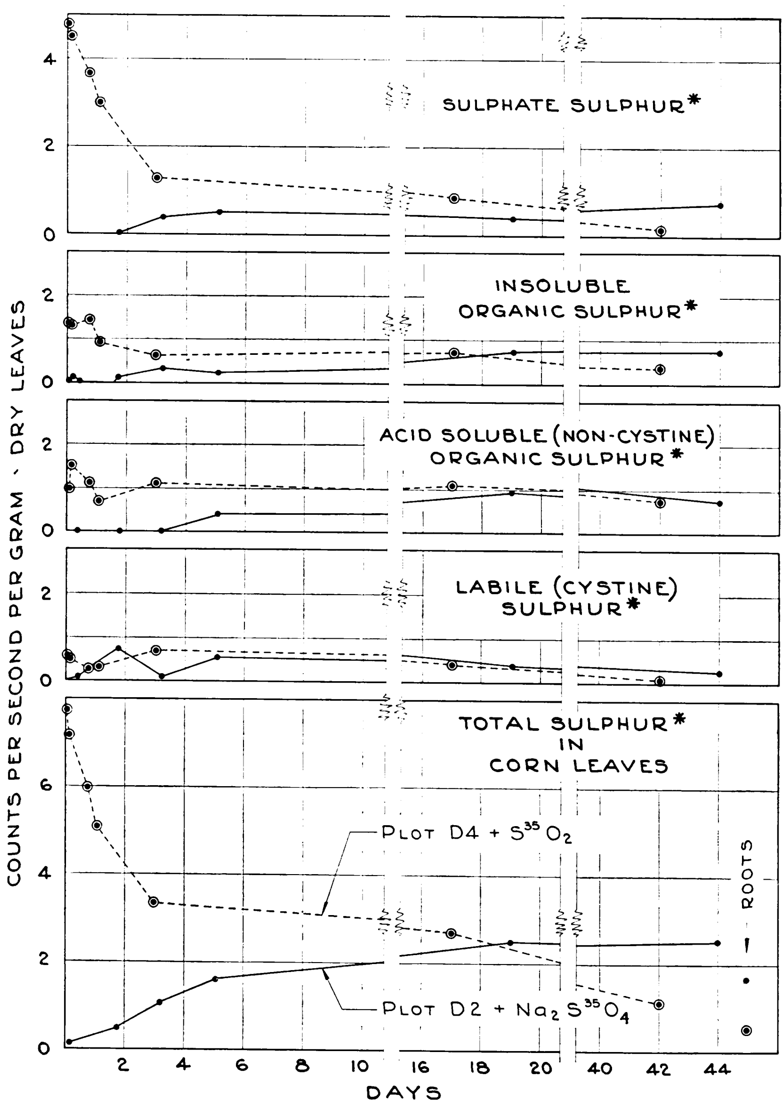

FIG. 2. Radiosulphur in the leaves of corn, adequately supplied with sulphate, at various times after treating plots D–2 and D–4 with sodium sulphate* and sulphur* dioxide, respectively. Fractionation of the leaf sulphur* is shown.

and insoluble organic sulphur*, and somewhat greater than the labile sulphur*. In the sodium sulphate* treatment of corn, all four fractions were roughly equal, though in the later samplings the labile fraction was somewhat smaller than the others.

TABLE IV

DISTRIBUTION OF RADIOSULPHUR IN THE WHEAT AND BARLEY PLANTS AT HARVEST (JULY 30, 1942) (COUNT PER SECOND PER GRAM DRY MATTER AND COUNT PER SECOND PER GRAM TIMES WEIGHT, OF DIFFERENT PARTS OF THE PLANTS)

PORTION TESTED	SULPHUR* DIOXIDE ADDED						SODIUM SULPHATE* ADDED					
	B-4 JUNE 6		A-3 JUNE 22		B-3 JUNE 29		B-2 JUNE 23		A-1 JUNE 23		B-1 JUNE 30	
	C/S/G	TOTAL C/S	C/S/G	TOTAL C/S	C/S/G	TOTAL C/S	C/S/G	TOTAL C/S	C/S/G	TOTAL C/S	C/S/G	TOTAL C/S
SPRING WHEAT												
Primary† grain	8.4	3200	10.2	3360	15.1	5600	23.2	6700	26.0	6220	23.4	8000
Secondary grain	7.7	410	9.3	1000	17.6	90	27.8	360	25.9	2100	25.2	100
Primary chaff	5.7	600	4.0	340	4.7	560	7.3	640	5.4	350	8.7	780
Secondary chaff	5.1	210	6.0	240	9.3	70	14.6	410	17.4	490	15.7	390
Primary straw	5.0	1930	5.6	1350	6.5	2500	5.8	1710	6.2	1160	6.9	2290
Secondary straw	3.5	290	7.2	590	11.7	470	16.2	1560	9.5	570	12.2	1320
Crowns	2.0	50	1.9	30	3.8	80	6.1	130	6.2	110	3.5	80
Roots	3.3	260	2.0	130	4.6	360	6.4	350	5.9	340	4.6	180
Total	....	6950	....	7040	....	9730	....	11860	....	11340	....	13140
BARLEY												
Primary grain	11.1	860	10.0	1050	7.0	1190	11.6	520	14.6	1330	10.0	720
Secondary grain	9.6	480	8.4	440	14.5	600	20.8	400	21.1	470	16.2	320
Tertiary grain	8.2	350	6.4	100	14.5	70	24.3	270	23.1	920	15.7	170
Primary chaff	5.7	280	5.2	110	4.5	250	7.7	150	8.3	180	6.2	230
Secondary chaff	4.8	110	5.7	90	7.3	190	7.7	260	9.6	80	6.6	200
Tertiary chaff	5.4	360	4.1	170	8.6	360	13.9	700	9.7	150	12.3	580
Primary straw	5.3	640	4.7	270	6.6	1080	2.8	170	2.7	160	2.8	230
Secondary straw	3.9	260	7.5	270	9.3	550	3.2	230	2.7	60	3.0	190
Tertiary straw	3.8	560	6.2	340	8.9	720	6.4	680	7.1	500	8.0	820
Quaternary straw	6.9	170	7.4	100	11.4	350	10.0	290	9.9	180	8.4	300
Crowns	3.3	50	2.7	30	3.1	40	4.8	60	Lost		6.3	270
Roots	4.8	160	3.5	60	5.0	180	7.7	250	6.2	160	5.1	290
Total	....	4280	....	3030	....	5580	....	3980	....	4190	....	4320

† "Primary," "Secondary," etc., refer to shoots of different ages.

TABLE IV—Continued

DISTRIBUTION OF RADIOSULPHUR IN THE WHEAT AND BARLEY PLANTS AT HARVEST (JULY 30, 1942) (COUNT PER SECOND PER GRAM DRY MATTER AND COUNT PER SECOND PER GRAM TIMES WEIGHT, OF DIFFERENT PARTS OF THE PLANTS)

PORTION TESTED	SULPHUR* DIOXIDE ADDED						SODIUM SULPHATE* ADDED					
	B-4 JUNE 6		A-3 JUNE 22		B-3 JUNE 29		B-2 JUNE 23		A-1 JUNE 23		B-1 JUNE 30	
	C/S/G	TOTAL C/S	C/S/G	TOTAL C/S	C/S/G	TOTAL C/S	C/S/G	TOTAL C/S	C/S/G	TOTAL C/S	C/S/G	TOTAL C/S
WINTER WHEAT												
Senescent‡ leaves	4.4	1130	9.6	1110	5.5	1340	2.9	390	3.6	200	3.1	470
Old leaves	3.5	520	9.3	1380	7.6	730	13.8	1260	17.5	1800	12.5	1210
Intermediate leaves	3.9	2000	5.8	2760	7.9	3590	16.9	6060	17.0	4470	14.4	5270
Young leaves	4.2	2620	6.6	2460	6.8	2690	13.3	4180	13.7	2980	11.6	3300
Crowns	3.3	320	4.8	480	8.1	820	11.0	1040	10.4	970	7.4	480
Roots	3.9	640	5.1	1400	3.9	820	8.2	1760	7.4	1330	6.5	1230
Total	....	7230	....	9590	....	9990	....	14690	....	11750	....	11960
Plot Total	....	18460	....	19660	....	25300	....	30530	....	27280	....	29420

‡ "Senescent," "Old," etc., refer to leaves of different ages on the individual shoots.

Distribution of radiosulphur at harvest

Table IV summarizes "count" data for the various plant structures of spring wheat, barley, and winter wheat at harvest. Particularly striking is the fact that the grain samples showed much greater activity than the other parts of the plants: in some cases 4 to 8 times as much activity per gram of dry matter as the straw. The least active portions of the plants were the crowns and roots. In the two early fumigations, the primary grain had more sulphur* per gram than the secondary, but the reverse was true in plot B–3 which received its fumigation when the primary heads were well established. In the sodium sulphate* treated plots, as a result of the more protracted additions of sulphur*, the secondary heads acquired a greater concentration than the primary in nearly all cases. The differences were small in the spring wheat but rather large in the barley which, as pointed out earlier, developed and matured ahead of the wheat. The weight of the primary grain, however, was so much larger than that of the later growth that the greatest total activity was always found in the primary grain. In the straw samples, the largest concentrations of sulphur* were nearly always found in the youngest tissues. It is probable that translocation from this tissue had not proceeded so far as in the older culms.

The distribution of the sulphur* in the winter wheat was fairly uniform except that there was considerably more activity in the tops than in the roots in all the treatments after the earliest one. The senescent leaves of the two earliest treatments had relatively more activity than those of the later treatments, indicating less absorption in the latter, due to the fact that the lower leaves began to dry up late in June.

Another method of showing the distribution of the sulphur* in the wheat and barley is employed in table V, to indicate differences between grain, chaff, straw, and roots. The table gives the percentage of the total plant weight and the percentage of the total count in each fraction and also the ratio of the two percentages. Thus, in the grain, the relative activity per unit weight is considerably greater than unity; in the chaff, about unity; and in the straw and roots, considerably less than unity. Translocation of the activity to the grain is therefore clearly indicated, since of course, the original absorption was principally in the vegetative portions of the plants.

The distribution of sulphur* in the corn plants, table VI, indicates that the greatest concentrations were found in the leaves and the lowest in the stalks and husks. These plants were still green at harvest and the ears were small and incompletely filled with kernels, due to incomplete fertilization in the rather calm atmosphere of the plant chambers. Evidently the ripening process did not advance sufficiently to induce appreciable translocation into the kernels. Neither the silks nor the tassels had as much sulphur* as the leaves.

A combined distribution and fractionation study of the activity of spring wheat in plot B–1 one week before harvest and in plots B–1 and B–3 after harvest is summarized in table VII. In the ripening plant on July 22, the

TABLE V

DISTRIBUTION OF RADIOSULPHUR IN WHEAT AND BARLEY PLANTS AT HARVEST (PERCENTAGE OF TOTAL COUNT DIVIDED BY PERCENTAGE OF TOTAL WEIGHT IN THE DIFFERENT PARTS OF THE PLANTS)

Plot	Treatment	Date	Grain			Chaff			Straw			Roots		
			Weight	Count	Count/Weight	Weight	Count	Count/Weight	Weight	Count	Count/Weight	Weight	Count	Count/Weight
			%	%		%	%		%	%		%	%	
						Spring wheat								
B-4	$S^{35}O_2$	June 6	37	51	1.38	13	12	0.92	43	33	0.77	7	4	0.57
A-3	"	" 22	45	62	1.38	13	8	0.62	36	28	0.78	6	2	0.33
B-3	"	" 29	37	58	1.57	12	6	0.50	43	32	0.75	8	4	0.50
B-2	$Na_2S^{35}O_4$	" 23	34	59	1.74	13	9	0.69	47	29	0.62	6	3	0.50
A-1	"	" 23	44	73	1.67	12	7	0.58	36	17	0.47	8	3	0.38
B-1	"	" 30	36	61	1.70	12	9	0.75	48	29	0.60	4	1	0.25
		Ave.......	39	61	1.57	12	8	0.68	42	28	0.67	7	3	0.43
						Barley								
B-4	$S^{35}O_2$	June 6	24	39	1.62	19	18	0.95	53	39	0.74	4	4	1.00
A-3	"	" 22	39	53	1.36	18	12	0.67	39	33	0.84	4	2	0.50
B-3	"	" 29	30	33	1.10	17	14	0.82	47	50	1.06	5	3	0.60
B-2	$Na_2S^{35}O_4$	" 23	15	30	2.00	21	28	1.33	57	36	0.63	7	6	0.86
A-1	"	" 23	39	65	1.67	11	10	0.91	43	21	0.49	7	4	0.57
B-1	"	" 30	17	28	1.65	19	23	1.21	55	42	0.76	9	7	0.78
		Ave.......	27	41	1.52	18	18	1.00	49	37	0.76	6	4	0.67
						Winter wheat			Leaves and crowns					
B-4	$S^{35}O_2$	June 6	....	....	....	....	....	....	90	91	1.01	10	9	0.90
A-3	"	" 22	....	....	....	....	....	....	80	85	1.06	20	15	0.75
B-3	"	" 29	....	....	....	....	....	....	87	92	1.06	13	8	0.62
B-2	$Na_2S^{35}O_4$	" 23	....	....	....	....	....	....	82	88	1.07	18	12	0.67
A-1	"	" 23	....	....	....	....	....	....	80	89	1.11	20	11	0.55
B-1	"	" 30	....	....	....	....	....	....	83	90	1.09	17	10	0.59
		Ave.......	....	....	....	....	....	....	84	89	1.06	16	11	0.69

TABLE VI

DISTRIBUTION OF TOTAL SULPHUR AND RADIOSULPHUR IN CORN PLANTS AT HARVEST ON OCTOBER 30, 1942

Portions tested	Plot D-4†				Plot D-2††			
	Dry weight	Total sulphur	Count per second		Dry weight	Total sulphur	Count per second	
			Per gram	Total			Per gram	Total
	gm.	%			gm.	%		
Upper 3 leaves	38	0.41	1.1	42	32	0.33	3.3	106
4th leaf	21	0.34	1.7	36	17	0.29	2.1	36
Leaves on ears	26	0.34	1.5	39	32	0.28	2.3	75
Tassels	23	0.24	1.1	25	20	0.21	1.9	38
Silks	22	0.17	0.4	9	20	0.17	1.5	30
Kernels	22	0.24	0.3	7	38	0.22	1.8	68
Cobs	87	0.18	0.3	26	141	0.14	1.6	226
Husks	176	0.10	0.1	18	138	0.09	0.5	69
Stalks	406	0.11	0.1	41	400	0.10	0.9	360
Roots	98	0.34	0.5	49	108	0.27	1.7	184
Total	919	....	....	292	946	....	....	1192

† Treated with sulphur* dioxide on Sept. 18.
†† Treated with sodium sulphate* on Sept. 16.

TABLE VII

DISTRIBUTION AND FRACTIONATION OF THE RADIOSULPHUR IN SPRING WHEAT ONE WEEK BEFORE HARVEST AND AT HARVEST

Plot	Date		Tissue	Radiosulphur in different parts of plants				
				Count	Recovered as			
	Treatment	Sampling		c/s/g	Labile S	Acid soluble organic S	Insoluble organic S	Sulphate S
					%	%	%	%
B-1	June 30	July 22†	Heads	11.8	31	20	30	17
		"	Leaves	13.0	11	31	39	19
		"	Sheaths	4.9	9	36	26	29
		"	Stems	5.7	6	19	14	61
B-1	June 30	July 30††	Primary grain	23.4	35	45	9	11
B-3	" 29	"	" "	15.1	44	40	9	7
B-1	June 30	July 30††	Secondary grain	25.2	30	48	9	13
B-3	" 29	"	" "	17.6	31	51	6	12
B-1	June 30	July 30††	Primary straw	6.9	10	34	20	36
B-3	" 29	"	" "	6.3	12	40	22	26
B-1	June 30	July 30††	Secondary straw	11.3	11	37	31	21
B-3	" 29	"	" "	10.8	4	46	34	16
B-1	June 30	July 30††	Roots	5.0	15	26	50	9
B-3	" 29	"	"	4.6	15	24	51	10

† Fresh samples—partly green.
†† Dried samples after final harvest.

TABLE VIII

FRACTIONATION OF RADIOSULPHUR IN WHEAT AND BARLEY KERNELS AFTER HARVEST

Plot	Primary grain					Secondary grain					Tertiary grain				
	Count per second per gm.	Sulphur* recovered as				Count per second per gm.	Sulphur* recovered as				Count per second per gm.	Sulphur* recovered as			
		Labile S	Acid soluble organic S	Insoluble organic S	Sulphate S		Labile S	Acid soluble organic S	Insoluble organic S	Sulphate S		Labile S	Acid soluble organic S	Insoluble organic S	Sulphate S
		%	%	%	%		%	%	%	%		%	%	%	%
Spring wheat															
B-4	8.4	28	48	20	4	7.7	30	52	11	7	....	......	....	....	......
A-3	10.2	42	39	12	7	9.3	39	44	10	7	....	......	....	....	......
B-3	15.1	44	40	9	7	17.6	31	51	6	12	....	......	....	....	......
Ave.....	11.2	38	43	14	6	11.5	33	49	9	9	....	......	....	....	......
B-2	23.2	37	45	10	8	27.8	35	45	14	6	....	......	....	....	......
A-1	26.0	43	39	11	7	25.9	44	36	12	8	....	......	....	....	......
B-1	23.4	38	43	10	9	25.2	30	49	9	13	....	......	....	....	......
Ave.....	24.2	38	43	10	9	26.3	36	43	12	9	....	......	....	....	......
Barley															
B-4	11.1	25	55	15	5	9.6	23	56	15	6	8.2	29	42	25	4
A-3	10.0	45	36	16	3	8.4	32	44	21	3	6.4	25	50	22	3
B-3	7.0	52	28	17	3	14.5	51	26	17	6	14.5	30	46	14	10
Ave.....	9.4	41	40	16	4	10.9	35	42	18	5	9.7	28	46	20	6
B-2	11.6	39	35	18	8	20.8	39	37	16	8	24.3	41	34	18	7
A-1	14.6	35	44	18	3	21.1	53	30	15	2	23.1	35	34	26	5
B-1	10.0	47	23	19	11	16.2	44	26	17	13	15.7	30	41	22	7
Ave.....	12.1	40	34	18	7	19.4	45	31	16	8	21.0	35	36	22	6

concentration of labile sulphur* was greater in the heads than in the rest of the plant; the concentrations of the soluble and insoluble organic sulphur* were greatest in the leaves and least in the stems; while the concentration of sulphate* was greatest in the stems and least in the heads. In fact, 60 per cent. of the total sulphur* in the stems was sulphate*. These relationships suggest that the organic sulphur* in the leaves was changed to sulphate* in order to translocate it to the heads where it was converted back to organic combination. The labile (cystine) fraction was increased greatly as a result of this transfer.

After harvest, the grain differed from the heads on July 22, principally in having less insoluble organic sulphur*. This was probably due in part to the fact that the heads of July 22 included the chaff, etc., which was high in the insoluble fraction. The straw still contained appreciable sulphate*, presumably in the stems, and was low in labile sulphur*. The roots were particularly high in insoluble organic sulphur*, and low in labile and sulphate sulphur*.

Table VIII presents the fractionation analyses of all grain samples from the spring wheat and barley. Labile and acid soluble organic sulphur* made up about 80 per cent. of the sulphur* in the spring wheat and about 70 to 80 per cent. of that in the barley; insoluble organic sulphur* accounted for 9 to 26 per cent. and sulphate* for only 3 to 13 per cent. There was some variability in the proportions of labile and soluble organic sulphur*, but it is not clear that any significance can be attached to the differences. Soluble organic sulphur was considerably higher than labile sulphur in all samples of plot B–4 which received the earliest treatment. The same relationship usually was found in the secondary wheat and tertiary barley but the reverse relationship characterized most of the other samples.

Discussion

It may be noted that the sodium sulphate* and sulphur* dioxide treatments usually gave similar results in corresponding plots. One striking fact is that nearly all the sulphate* values were low. This was not expected in the fumigated plots because they had extensive treatments with inactive sulphur dioxide throughout the growth period as already described. In previous work with alfalfa, fumigation treatments of this kind raised markedly the sulphur content of the vegetation and the increase was found to be due largely to sulphate. In these grain plots it has been shown by analysis of the plant tissue (tables II and VI) that the total sulphur values of the fumigated and unfumigated plants were not appreciably different and accordingly there should be no appreciable difference between the sulphate* values of the sodium sulphate* and sulphur* dioxide treated plots due to the long fumigations with inactive sulphur dioxide.

In the preceding paper (12) it was shown that the labile sulphur in the fumigated alfalfa leaves was generally higher than in the unfumigated leaves. The amount of the excess was greatest in the sulphur-deficient plots. Likewise there was a tendency for the soluble and insoluble organic matter to be higher in the fumigated than in the unfumigated plots, though the differences were small and not highly significant statistically. The present study of grain with radiosulphur, indicates that all three organic sulphur fractions were definitely formed from sulphur dioxide. It may therefore be concluded from the analytical data for alfalfa in the preceding paper that—though the amounts were small—some sulphur dioxide was reduced to each of the organic sulphur fractions. The similarity in the behavior of the sulphur* from the two sources indicates that sulphur nutrition by sulphur dioxide follows approximately the same course chemically as nutrition by sulphate.

Summary

Mixed plots of wheat and barley growing in nutrient solution deficient in sulphate, and corn plants growing in nutrient solution adequately supplied with sulphur, were either treated with radioactive sodium sulphate* in the nutrient solution or were fumigated with active sulphur* dioxide.

The results with sulphur-deficient wheat and barley indicate:

1. High initial absorption of sulphur* dioxide occurred in the leaves, followed by a lowering of the concentration as the sulphur* was distributed throughout the plant.

2. There was rapid absorption of sodium sulphate* from the nutrient solution, to build up a maximum concentration in the leaves in about 8 to 10 days, followed by steady lowering of the concentration as this sulphur* also was distributed.

3. Concentration in the tops was greater than in the roots.

4. Translocation to the grain of 60 to 80 per cent. of the sulphur* occurred during ripening.

5. Conversion of most of the sulphur* dioxide and sulphate* to organic forms occurred rapidly.

6. In the leaves the acid soluble and acid insoluble organic fractions predominated, but appreciable amounts of labile and sulphate sulphur* were also present; in the other parts of the plant the predominating fractions were as follows: stems, sulphate; roots, the acid insoluble fraction; and kernels, the labile and acid soluble fraction. There was only a little of the acid insoluble fraction and sulphate* in the kernels.

7. Evidently the organic sulphur* in the leaves was changed to sulphate for purposes of translocation; then changed back again to organic forms in the roots and grain. In this transfer to the grain, the labile fraction was greatly increased.

8. In corn, growing in a nutrient solution adequately supplied with sulphate, absorption of active sulphate* was much slower than in the wheat and barley, growing in sulphate-deficient solution.

9. Higher concentrations of sulphate* were generally present in the corn than in the wheat or barley. Sulphate* was a particularly important constituent of the corn leaves for several days after the sulphur* dioxide fumigation.

10. Greatest concentration of sulphur* was found in the leaves of corn; the least in the stalks and husks. The ripening process, however, was not far advanced at the end of the season, and the distribution at maturity could not be established.

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